Dietary Flexibility and Intestinal Plasticity in Birds: A Field and Laboratory Study

Pablo Sabat[1]
Fernando Novoa[1]
Francisco Bozinovic[2]
Carlos Martínez del Rio[3,*]
[1]Departamento de Ciencias Ecológicas, Facultad de Ciencias, Universidad de Chile, Casilla 653, Santiago, Chile; [2]Departamento de Ecologia, Facultad de Ciencias Biologicas, P. Universidad Catolica de Chile, Casilla 114-D, Santiago, Chile; [3]Department of Zoology and Physiology, University of Wyoming, Laramie, Wyoming 82071-3166

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ABSTRACT

The adaptive modulation hypothesis posits that the expression of digestive proteins should be modulated in response to intake of their respective substrates. A corollary of this hypothesis suggests that dietary flexibility and digestive plasticity should be correlated. We examined these two hypotheses in two granivorous Chilean birds (*Zonotrichia capensis* and *Diuca diuca*) that differ in dietary breadth. *D. diuca* is a strict granivore, whereas *Z. capensis* also eats insects. In field-caught birds, the activity of the intestinal dipeptidase aminopeptidase-N was positively correlated with intake of insects in *Z. capensis* but not in *D. diuca*. This is the first field documentation of modulation of intestinal enzymes by diet in birds. Intestinal maltase and sucrase activities were not correlated with seed (vs. insect) intake in either species. In the laboratory, captive birds of both species exhibited similar modulation of membrane-bound intestinal hydrolases when fed on synthetic diets of contrasting carbohydrate and protein composition. Maltase, sucrase, and aminopeptidase-N activities were significantly higher in birds fed on the carbohydrate-free than those on the carbohydrate-containing diet. Activities of the three enzymes were positively correlated. Therefore, this increase probably resulted from nonspecific increases of all enzymes resulting from intake of the carbohydrate-free diet. Principal components analysis separating the effect of diet on specific and on nonspecific modulation revealed that diet had a strong effect on nonspecific activity of intestinal enzymes in both *Z. capensis* and *D. diuca*. Diet also significantly affected aminopeptidase-N activities when the effect of diet on nonspecific modulation was removed. Birds fed on the carbohydrate-free, high-protein diet had significantly higher specific aminopeptidase-N activities than those fed on the carbohydrate-containing diet. Our results cast doubts on the notion that dietary flexibility and the plasticity of the gut's enzymes are necessarily correlated and on the general validity of the adaptive modulation hypothesis.

Introduction

The study of digestive processes has the potential of providing a mechanistic bridge between the study of physiological processes taking place within the digestive tract and feeding ecology. The design and adaptability of the gut may determine diet diversity and hence niche width (Karasov 1996), and digestion rates may influence feeding rates, time-budget allocation, and ultimately the rate at which animals acquire energy and essential nutrients (Kersten and Visser 1996). Because the digestive tract appears to show remarkable lability in phenotypic expression both within and among individuals (Diamond 1991), the study of digestion may be relevant to understanding the evolution of phenotypic plasticity, which is a central topic in evolutionary biology (Pigliucci 1996).

Here we examine the relationship between physiological plasticity and behavioral and ecological flexibility (Buddington et al. 1991; Diamond and Hammond 1992). Throughout the text, we use the term "digestive plasticity" to signify the ability to change the morphology and modulate the biochemistry of the gut in response to changes in diet. Digestive plasticity is an example of a flexible (sensu Stearns [1989]) or reversible (sensu Travis [1994]) norm of reaction. We use the term "dietary flexibility" to denote the magnitude of diversity in feeding habits. Our study compares the intestinal digestive plasticity of two species of granivorous birds with contrasting dietary flexibilities. *Zonotrichia capensis* (rufous-collared sparrow, Fringillidae, Emberizinae; our taxonomy follows Sibley and Monroe [1990]) is a widespread, granivorous species that includes significant amounts of insects in its diet, and *Diuca diuca* (common diuca finch, Fringillidae, Emberizinae) is a strict granivore (López-Calleja 1995). We explored the hypothesis that dietary flexibility is correlated with digestive plasticity

*To whom correspondence should be addressed. Present address: Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, Arizona 85721-0088; E-mail: cdelrio@u.arizona.edu.

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and predicted that *Z. capensis* would exhibit more digestive plasticity both in the laboratory and in the field than *D. diuca.*

Several studies have examined the digestive responses of birds to diets of contrasting nutrient composition in the laboratory (reviewed by Karasov [1996]). To our knowledge, no study has documented temporal variation in gut biochemistry (e.g., activity of digestive enzyme and nutrient transporters) in wild free-living birds (see Sabat and Bozinovic [1994] for an example on an insectivorous marsupial). Our study contains two complementary sections. In the first section, we document changes in diet and intestinal morphology and biochemistry in wild birds in the field throughout the year. In the second, we examine the digestive responses of the intestine of birds to diets of contrasting nutrient composition in the more controlled conditions of the laboratory.

Changes in the morphology of bird intestine in response to variation in diet and energy demands have been examined in both the laboratory and the field (reviewed by Karasov [1996]). A broad conclusion of these studies is that the size of the intestine correlates with food intake. Conditions favoring hyperphagia, such as cold exposure and chronic ingestion of low-quality food, lead to increased intestinal mass (reviewed by Karasov [1996]). Increased intestinal size appears to allow birds to process a large amount of food without compromising exposure time of ingested particles to digestive processes and extraction efficiency (Karasov 1996). Changes in intestinal biochemistry have been examined primarily in the laboratory by exposing birds to diets with contrasting nutrient compositions. Birds can maintain overall extraction efficiency when confronted with diets of contrasting composition by matching rates of hydrolysis and uptake of different nutrients with their contents in diet (reviewed by Karasov [1996]).

In this study, we examined the effect of diet on the activity of three intestinal membrane-bound digestive enzymes: the two disaccharidases sucrase (EC 3.2.1.48) and maltase (EC 3.2.1.20), and the oligopeptidase aminopeptidase-N (EC 3.4.11.2; also called leucine-aminopeptidase or amino-oligopeptidase; Vonk and Western 1984). We chose the disaccharidases as indicators of a bird's ability to assimilate carbohydrates and the oligopeptidase as an indicator of protein digestion. We expected increased activity of disaccharidases in birds ingesting seed diets in the field and high-carbohydrate diets in the laboratory. In contrast, we expected the activity of aminopeptidase-N to increase with the insect content in a bird's diet and with laboratory diets with high protein contents.

Diamond (1991) has argued that unused proteins in the brush border membrane of intestinal cells may take up much-needed space and may be costly in terms of biosynthetic energy and limiting nutrients in the face of rapid turnover. Thus, we expected a trade-off in the ability to efficiently utilize either protein-rich insect diets or carbohydrate-rich seed diets. As evidence for this trade-off, we hypothesized a negative correlation between the activity of intestinal disaccharidases and aminopeptidase-N.

Material and Methods

Field Methods and Study Site

Birds were captured at El Pangue (central Chile; 33°17′ S, 71°11′ W). The climate of El Pangue is Mediterranean, with warm, dry summers and cool, moist winters. The rainy season takes place from June to September, and annual precipitation averages 500 mm. At El Pangue, *Zonotrichia capensis* and *Diuca diuca* individuals often feed on the ground in mixed flocks. Birds were captured in agricultural fields with mist nets from December 1994 to September 1995. Eight to 10 birds were captured bimonthly, with the exception of November 1994, when 10 *Z. capensis* individuals but no *D. diuca* were captured. Birds were killed by thoracic compression (American Ornithologists' Union 1988) within 30 min of capture. The intestine was immediately excised, flushed with ice-cold saline (1.02%), and measured and weighed before storage in liquid nitrogen for subsequent enzyme activity measurements. The contents of the crop, proventriculus, and gizzard were stored in vials for posterior diet analysis. For diet analysis, we determined the volumetric percentage of insects and seeds found in each bird's upper gastrointestinal tract. To satisfy normality assumptions of parametric statistical tests (Zar 1996), we arcsin square root transformed percentage data before analyses.

Laboratory Trials

Sixteen birds of each species were captured in March 1995. Birds in the laboratory were housed individually in 60 × 60 × 60 cm cages, under a 12D : 12L cycle and at an average temperature of 21°C. During a captivity acclimation period lasting 30 d, birds were provided with water and a seed mixture and mealworms ad lib. After this acclimation period, each bird was assigned to one of two isocaloric synthetic diets: a carbohydrate-free diet or a diet containing 52.2% carbohydrate. In the carbohydrate-free diet, protein replaced carbohydrate. Our experimental diets were identical to those described by Biviano et al. (1993), except that we used egg albumin instead of soy protein isolate as a protein source. Itemized descriptions of our experimental diets are presented in Biviano et al. (1993) and Martínez del Rio et al. (1995). Birds were maintained on artificial diets for 20 d, after which their intestines were processed and stored for enzyme measurements as described above for birds captured in the field. Because some individuals did not adjust well to captivity and others refused to eat the synthetic diets, the number of individuals on the carbohydrate and carbohydrate-free treatments were seven and six for *Z. capensis* and *D. diuca,* respectively.

Enzyme Activity Measurements

Small intestines were thawed at 5°C and homogenized (30 s, OMNI 5000 homogenizer at setting 6) in nine volumes of

350 mmol L^{-1} mannitol in 1 mmol L^{-1} Hepes/KOH, pH 7.5. Disaccharidase activities were measured following Martínez del Rio et al. (1995). In brief, tissue homogenates (100 μL) diluted with 350 mmol L^{-1} mannitol in 1 mmol L^{-1} Hepes/KOH were incubated at 40°C with 100 μL of 56 mmol L^{-1} sugar (sucrose or maltose) solutions in 0.1 mol L^{-1} maleate/NaOH buffer, pH 6.5. After a 10–20-min incubation, reactions were arrested by adding 3 mL of a stop/developing Glucose-Trinder (one bottle of Glucose-Trinder 500 reagent [Sigma, St. Louis, Mo.] in 250 mL 1.0 mol L^{-1} TRIS/HCl, pH 7, plus 250 mL of 0.5 mol L^{-1} NaH_2PO_4/Na_2HPO_4, pH 7). After 18 min at 20°C, absorbance of the resulting solution was measured at 505 nm with a Beckman DU-64 spectrophotometer. In our preparation, disaccharide hydrolyses were linear even after 30 min. Apparent Michaelis constants and pH optima for intestinal maltase and sucrase activity of *Z. capensis* and *D. diuca* are presented in Figures 1 and 2.

Aminopeptidase-N assays were done using L-alanine-p-nitroanilide as a substrate. In brief, 100 μL of homogenate diluted with mannitol/KOH buffer were mixed with 1 mL of a prewarmed (40°C) assay mix (2.04 mmol L^{-1} L-alanine-p-nitroanilide in 0.2 mol L^{-1} NaH_2PO4/Na_2HPO_4, pH 7). The reaction was incubated at 40°C and arrested after 10 min with 3 mL of ice-cold 2 N acetic acid, and absorbance was measured at 384 nm. The hydrolysis of L-alanine-p-nitroanilide was linear for up to 20 min in our preparations. Apparent Michaelis constants and pH optima for intestinal aminopeptidase-N in *Z. capensis* and *D. diuca* are presented in Figures 1 and 2.

On the basis of absorbance standards constructed for glucose and p-nitroanilide, we calculated total and standardized intestinal activities. Thus, data on enzyme activities are presented as total hydrolytic activity (μmol min^{-1}) and activity per unit intestinal wet mass (μmol min^{-1} mg^{-1} wet tissue). Martínez del Rio et al. (1995) provide justification for our choice of standardization. In brief, we conducted assays in homogenates because the enrichment of brush border membrane vesicle preparations differs significantly among batches, and because brush border membrane vesicle preparations tend to have low and variable yields (Martínez del Rio et al. [1995] and references therein). These two factors increase the variability of estimates of intestinal enzyme activities from brush border membrane vesicle preparations and thus hinder intra- and interspecific comparisons.

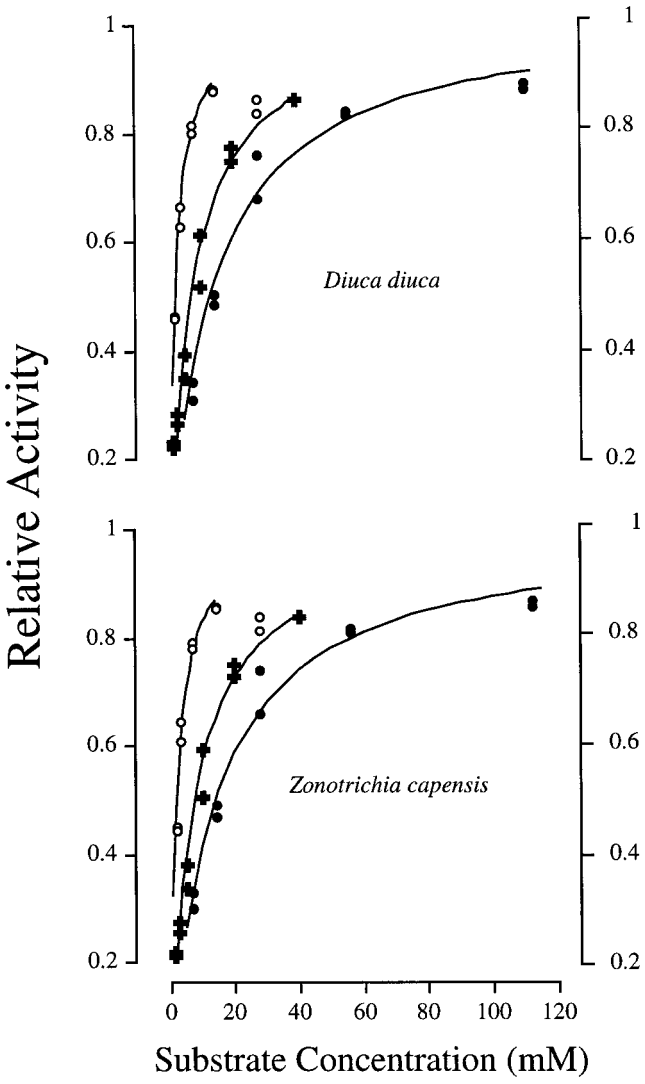

Figure 1. Hydrolysis of maltose (*open circles*), sucrose (*closed circles*), and L-alanine-p-nitroanilide (*crosses*) as a function of substrate concentration by intestinal homogenates of *Zonotrichia capensis* and *Diuca diuca*. Curves were fitted with a nonlinear least squares procedure (Motulsky and Ransnas 1987). Hydrolysis values are normalized to maximal hydrolytic rate. The apparent Michaelis constants (mean ± SE for *Z. capensis* and *D. diuca*, respectively) are: maltase, 1.60 ± 0.07, 2.08 ± 0.06; sucrase, 10.96 ± 0.52, 14.04 ± 0.72; and aminopeptidase-N, 5.25 ± 0.37, 7.5 ± 0.53. Because maltase activity exhibits substrate inhibition, kinetic parameters were fitted for maltose concentrations lower than 20 mmol L^{-1}.

Results

Diet, Intestinal Morphology, and Enzyme Activities in the Field

Diuca diuca fed almost exclusively on seeds throughout the year (mean ± SD = 99.1% ± 3.3% of gut contents, $n = 47$), with no significant month-to-month differences (ANOVA on arcsin square root transformed data, $F_{4, 41} = 0.51$, $P = 0.72$; Fig. 3). The interindividual variation in the diet of *D. diuca* was very small (average monthly coefficient of variation [CV] = 3.4%) and did not differ significantly among months (test for homogeneity of CV, $\chi^2 = 7.7$, $P > 0.1$; Zar 1996). In a similar fashion, neither the mean percentage intake of seeds nor the monthly coefficient of variation in percent seed intake of *Zonotrichia capensis* differed significantly among months (ANOVA on arcsin square root transformed data, $F_{5, 43} = 1.6$ $P = 0.18$, and test for homogeneity of CV, $\chi^2 = 7.7$, $P > 0.1$;

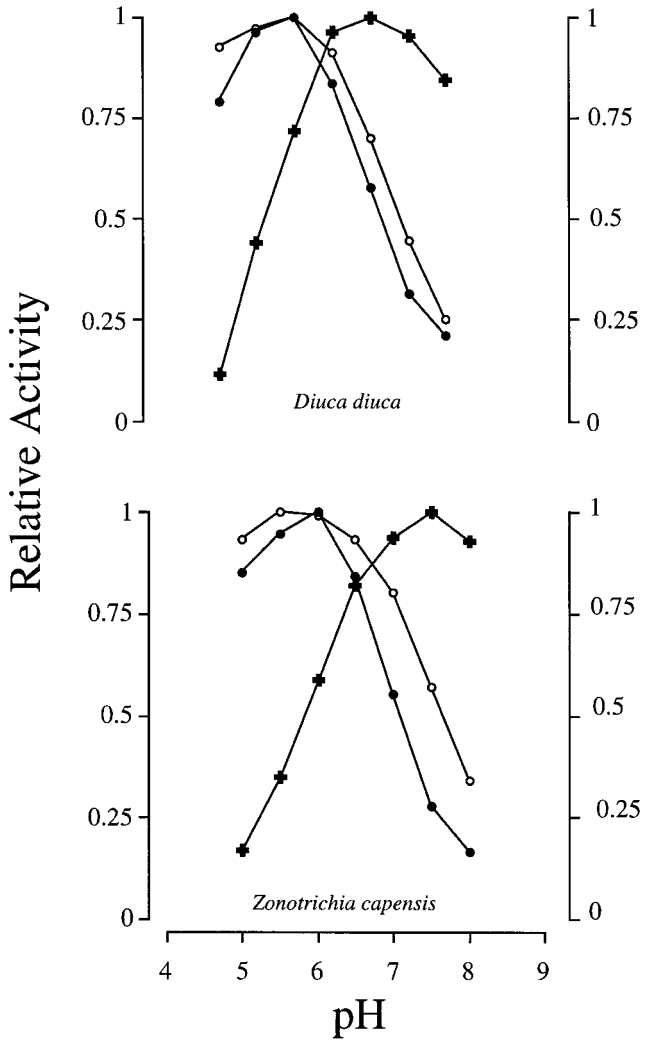

Figure 2. pH profiles for maltase (*open circles*), sucrase (*closed circles*), and aminopetidase-N (*crosses*) in *Zonotrichia capensis* and *Diuca diuca.* pH profiles were standardized to the maximum value measured.

Fig. 3). This species, however, included more insects in its diet than did *D. diuca* (mean % seeds ± SD = 78.5% ± 25.0%, $n = 52$; ANOVA on arcsin square root transformed data, $F_{1, 94} = 1{,}113$, $P < 0.001$) and exhibited roughly 10 times more interindividual monthly variation in diet (average monthly CV = 31.9%; $Z = 7.4$, $P < 0.01$; Zar 1996). Thus, although the degree of granivory versus insectivory did not vary significantly throughout the year for *D. diuca* and *Z. capensis,* these two species showed contrasting diets and, perhaps more important, dissimilar variability in diet.

The analyses described above rely on coefficient of variation as an estimator of dietary variation. The same patterns are found if variance in percent seed intake is used as an alternative estimator of dietary variation. *D. diuca* and *Z. capensis* did not show significant differences in dietary variance among months (Bartlett's tests for homogeneity of variances, $P > 0.1$), but variance in diet was significantly higher in *Z. capensis* than in *D. diuca* (pooled monthly variances for *D. diuca* and *Z. capensis* were 11.0 and 626.7, respectively; Bartlett's test, $P < 0.01$). Because statistical tests comparing estimates of variation are notoriously weak (Zar 1996), the differences in interindividual dietary variation among *Z. capensis* and *D. diuca* are robust.

Z. capensis did not exhibit significant differences among months in body or intestinal mass (mean body mass ± SD, 22.0 ± 1.2 g, ANOVA, $F_{5, 50} = 1.1$, $P > 0.1$; mean intestinal mass ± SD, 1.0 ± 0.2 g, ANOVA, $F_{5, 50} = 1.6$, $P > 0.1$). *D. diuca* individuals, in contrast, varied significantly in both body and intestinal mass throughout the year ($F_{4, 44} = 2.7$ and 2.8, $P < 0.05$, for body and intestinal mass, respectively; Fig. 4). *Z. capensis* showed significant variation in intestinal length throughout the year (ANOVA, $F_{5, 50} = 4.0$, $P > 0.01$), but *D. diuca* did not ($F_{4, 44} = 2.3$, $P > 0.05$). Because intestinal mass and body mass were not significantly correlated in *Z. capensis* or *D. diuca* ($r = 0.32$ and $r = 0.24$, for *Z. capensis* and *D. diuca,* respectively, $P > 0.1$ for both), we did not compare the variation in mass-specific intestinal mass throughout the year.

Total activity of all intestinal enzymes was tightly and positively correlated with activity standardized by intestinal mass

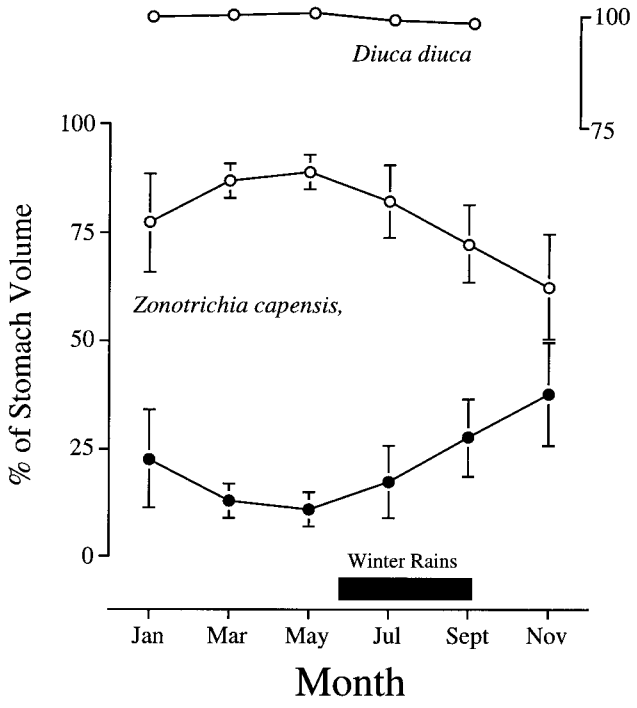

Figure 3. Temporal variation in the volumetric proportion of insects (*closed circles*) versus seeds (*open circles*) in the diets of *Zonotrichia capensis* and *Diuca diuca.* Points are means (*n* ranged from eight to 10 individuals), and bars are standard errors. Note that *D. diuca* showed an almost exclusively granivorous diet throughout the year, whereas *Z. capensis* includes a significant amount of insects in the diet.

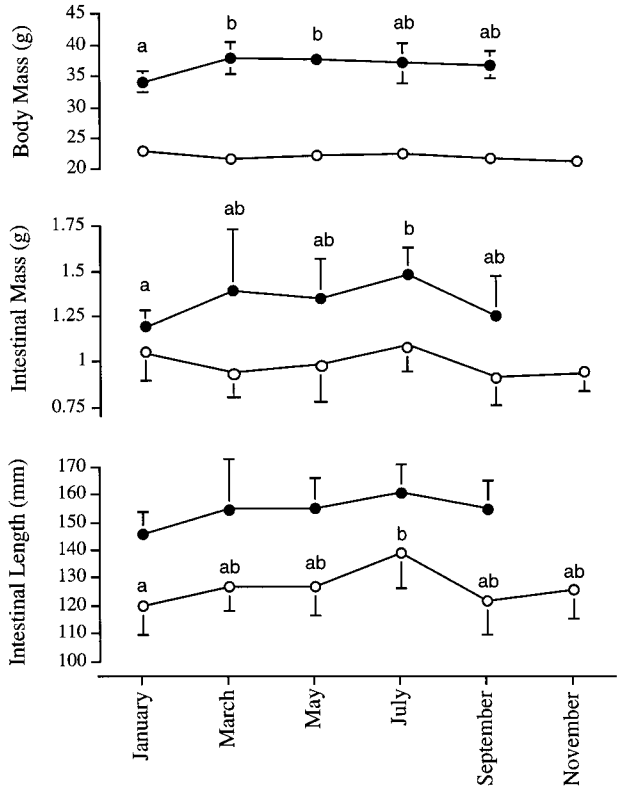

Figure 4. Temporal variation in body mass, intestinal mass, and intestinal length in *Zonotrichia capensis* (*open circles*) and *Diuca diuca* (*closed circles*). Points are means (*n* ranged from eight to 10 individuals), and bars are standard errors. Letters denote means that do not differ from each other from month to month after a Tukey's multiple-comparison test (Zar 1996). Lines without any letters show traits for which there was no significant variation among months.

($r > 0.75$; $n = 56$ and $n = 49$ for *Z. capensis* and *D. diuca,* respectively). Therefore, we present data for enzyme activity standardized by intestinal mass exclusively. Because the month-to-month average diet did not differ significantly for either species, rather than looking for temporal variation in enzyme activities, we explored the correlation between enzyme activities and an individual's diet. *D. diuca* did not show any significant correlations between insect versus seed percentage in diet and sucrase, maltase, or aminopeptidase-N activity ($r = 0.17$, $r = 0.029$, and $r = 0.012$, respectively, $P > 0.3$, $n = 46$ and $n = 52$). Given the exceedingly low variation in diet exhibited by this species, this result is not surprising. *Z. capensis* showed no correlation between percentage of insects in diet and the activities of the disaccharidases sucrase and maltase ($r = 0.09$ and $r = 0.01$, respectively, $P > 0.3$, $n = 56$). The activity of aminopeptidase-N, however, was strongly positively correlated with the percentage of insects in a bird's gut ($r = 0.44$, $P < 0.002$; Fig. 5).

Because *Z. capensis* exhibited significantly more interindividual variation in diet than *D. diuca,* it is of interest to ask whether this difference was accompanied by a concomitant difference in variation in enzyme expression. For the disaccharidases maltase and sucrase, variance and coefficients of variation were almost identical for *Z. capensis* and *D. diuca* ($P > 0.4$ after Bartlett's tests). In contrast, both coefficient of variation and variance in the expression of aminopeptidase-N activity were significantly higher in *Z. capensis* ($P < 0.02$; Table 1).

In disagreement with our prediction of a negative correlation between the activities of the disaccharidases sucrase and maltase, and aminopeptidase-N, we found strong positive correlations between sucrase and maltase activity and the intestinal activity of aminopeptidase-N in *D. diuca* ($r = 0.49$ and $r = 0.51$, respectively, $P < 0.001$; Fig. 6). We also found weak, albeit statistically significant, positive correlations between intestinal sucrase and maltase activity and aminopeptidase-N in *Z. capensis* ($r = 0.27$ and $r = 0.27$, respectively, $P < 0.05$; Fig. 6). The activities of the two disaccharidases sucrase and maltase were very tightly, linearly, and positively correlated in both *D. diuca* and *Z. capensis* ($r = 0.89$ and $r = 0.91$, respectively, $P < 0.001$).

Because the three enzymes studied showed positive correlations, it is difficult to ascertain whether the variation in their activities was the result of factors that led to changes in all of them (nonspecific modulation; Karasov and Diamond 1988) or factors that led to the activity of only some of them (specific modulation). To disentangle the contribution of nonspecific and specific modulation to the total variation in intestinal hydrolytic activity in the field, we used principal components analysis (PCA) using the activity of all enzymes standardized by wet mass as variables. PCA is a useful multivariate technique

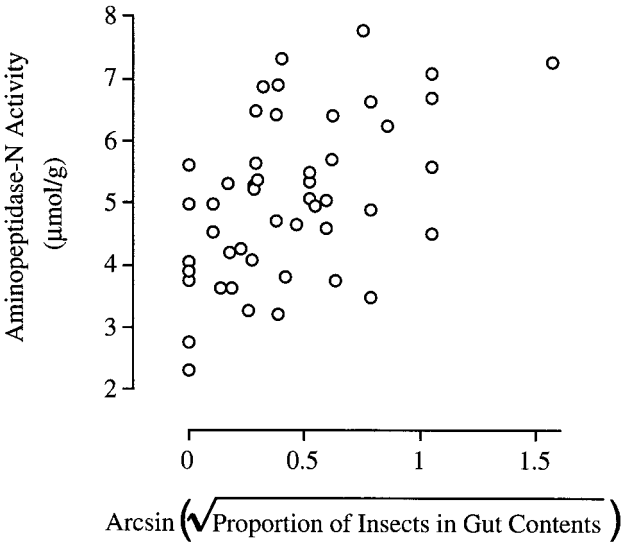

Figure 5. Relationship between aminopeptidase-N intestinal activity and volumetric proportion of insects in the gut. The positive correlation is highly significant ($r = 0.44$, $P < 0.002$).

Table 1: Variance in the activity (μmol min^{-1} g^{-1}) of intestinal hydrolases of *Zonotrichia capensis* ($n = 56$) and *Diuca diuca* ($n = 49$) captured in the field

	Maltase	Sucrase	Aminopeptidase-N
Zonotrichia capensis	181.99	1.63	1.99
Diuca diuca	181.46	1.36	.99
P	.55	.48	.02

Note. *P*-values are for Bartlett's tests (Zar 1996).

in this case because it reduces the number of correlated variables (enzyme activities) to a smaller number of uncorrelated variables that have a simple intuitive interpretation (Manly 1986). PCA reduced the three enzyme activities to two PCA axes that accounted for more than 96% of the variation in both *Z. capensis* and *D. diuca* (Table 2). In both species, the first component axis (PCA axis 1) was positively correlated with the activities of sucrase, maltase, and aminopeptidase-N. Thus, PCA axis 1 can be interpreted as a nonspecific activity axis. Note that PCA axis 1 accounts for a large amount of variation in enzyme activity in both *Z. capensis* and *D. diuca* (Table 2). It appears that most of the variation found in the intestinal hydrolytic activity of these two species (68% and 76%, respectively) in the field can be accounted for by nonspecific correlated variation in intestinal enzymatic activity. The second axis (PCA axis 2) was weakly negatively correlated with the activity of the two disaccharidases sucrase and maltase but was very strongly positively correlated with the activity of aminopeptidase-N (Table 2). PCA axis 2 can be interpreted as the residual activity of aminopeptidase-N that results when the effect of nonspecific variation is accounted for (specific aminopeptidase-N axis).

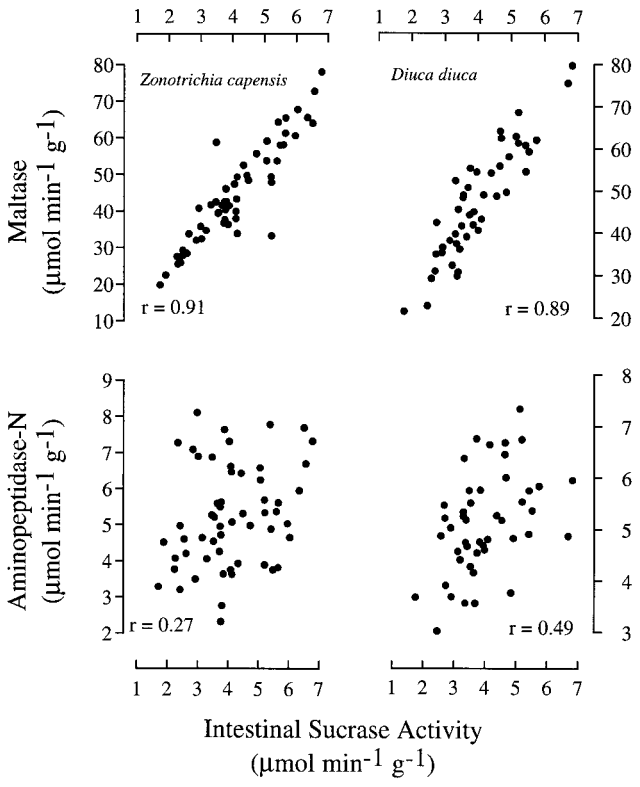

Figure 6. Relationship between intestinal sucrase activity and maltase and aminopeptidase-N activities in field-caught birds. Note that all the correlations shown are statistically significant ($P < 0.05$).

PCA axis 1 did not show positive correlations with the proportion of insects in the diet ($r < 0.12$, $P > 0.4$). Thus, the proportion of insects versus seeds in the diet did not seem to influence nonspecific modulation of intestinal hydrolytic activities in *Z. capensis* or *D. diuca*. PCA axis 2 was strongly correlated with the proportion of insects in the diet in *Z. capensis* ($r = 0.50$, $P < 0.003$) but not in *D. diuca* ($r = 0.038$, $P > 0.5$). Thus, PCA supported our previous analyses; it appears that in *Z. capensis* an increased proportion of insects in the diet led to upward specific modulation of aminopetidase-N.

Intestinal Morphology and Intestinal Enzyme Activities in the Laboratory

Z. capensis and *D. diuca* did not exhibit significant differences among experimental diets in body or intestinal mass (Table 3). Both species showed significant differences between diets in sucrase, maltase, and aminopeptidase-N (Table 3). Contrary to our expectations, the intestinal activity of the three enzymes was higher in the carbohydrate-free than in the carbohydrate-containing diet (Table 3; Fig. 7). Furthermore, as in the field study, the activities of the three intestinal enzymes were significantly positively correlated (Fig. 7). It appeared that the carbohydrate-free diet stimulated the activity of the three enzymes in a nonspecific fashion.

To disentangle the effect of our experimental diets on specific and nonspecific modulation of intestinal enzymes in the laboratory, we again conducted PCA. This analysis yielded two principal components that accounted for 97% and 98% of the variance in *Z. capensis* and *D. diuca,* respectively (Table 2). In both *Z. capensis* and *D. diuca,* the first principal component (PCA axis 1) explained most of the variance (80% and 81%,

Table 2: PCA axes derived from analysis of the activities of three intestinal digestive enzyme activities in *Zonotrichia capensis* and *Diuca diuca* in the field and laboratory

	Field		Laboratory	
	PCA Axis 1	PCA Axis 2	PCA Axis 1	PCA Axis 2
Zonotrichia capensis:				
Factor loadings:				
Sucrase	.95	−.23	.90	−.24
Maltase	.95	−.23	.92	−.24
Aminopeptidase-N	.50	.88	.85	.84
Eigenvalue	2.05	.86	2.39	.52
Variance explained (%)	68	29	80	17
Cumulative variance explained (%)	68	97	80	97
Diuca diuca:				
Factor loadings:				
Sucrase	.93	−.28	.93	−.22
Maltase	.93	−.25	.92	−.25
Aminopeptidase-N	.73	.68	.87	.53
Eigenvalue	2.30	.61	2.08	.51
Variance explained (%)	76	20	81	17
Cumulative variance explained (%)	76	96	81	98

Note. In the laboratory, birds were fed two synthetic equicaloric diets of contrasting carbohydrate content; see text for composition.

respectively) and was strongly positively correlated with the activity of all intestinal digestive enzymes (Table 2). As in the field study, we interpret PCA axis 1 as a nonspecific activity axis. The second principal component axis (PCA axis 2) explained a smaller amount of variance (17% for both *Z. capensis* and *D. diuca*) and was weakly and negatively correlated with the disaccharidases sucrase and maltase, but strongly and positively correlated with aminopeptidase-N (Table 2). As in the field study, we interpret PCA axis 2 as an aminopeptidase-N axis that results from variation in aminopeptidase-N when

Table 3: Comparison of body mass (g), intestinal mass (g), and sucrase, maltase, and aminopeptidase-N intestinal activities (μmol min^{-1} g^{-1}) for two species of granivorous birds fed on two synthetic diets

	Carbohydrate Diet	Carbohydrate-Free Diet	*F*	*P*
Zonotrichia capensis:				
Mass	21.6 ± 1.7	20.6 ± 1.3	1.47	.24
Intestinal mass	.85 ± .17	.94 ± .11	2.70	.12
Sucrase	2.64 ± .99	3.85 ± 1.09	4.80	.042
Maltase	26.48 ± 11.03	39.82 ± 10.71	5.15	.040
Aminopeptidase-N	5.39 ± 1.30	8.91 ± 1.91	16.82	.002
Diuca diuca:				
Mass	32.9 ± 1.3	32.7 ± 3.0	.31	.86
Intestinal mass	1.14 ± .36	1.29 ± .18	.88	.36
Sucrase	2.75 ± .99	4.60 ± .93	11.06	.008
Maltase	33.01 ± 13.23	51.42 ± 9.96	7.80	.018
Aminopeptidase-N	5.43 ± 1.30	9.37 ± 1.22	31.19	.001

Note. Values are means ± SD; $n = 13$.

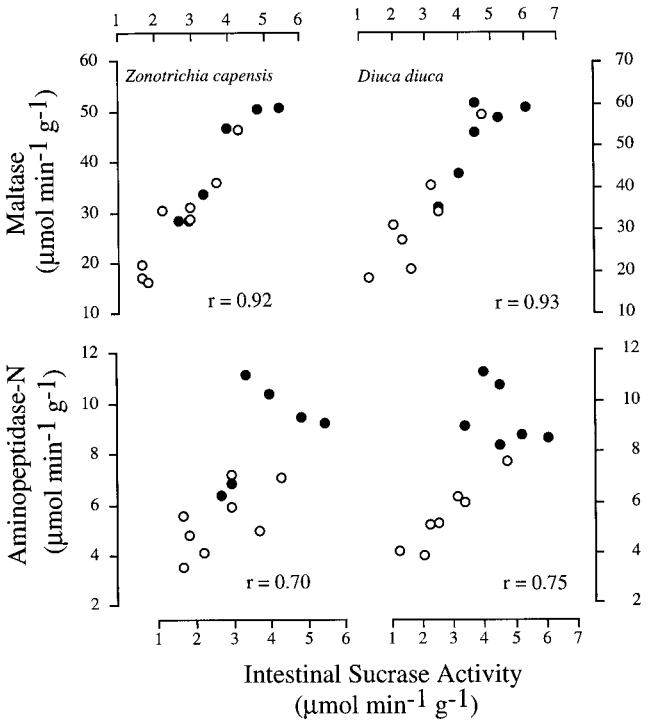

Figure 7. Relationship between intestinal sucrase activity and maltase and aminopeptidase-N activities in birds fed on artificial diets in the laboratory. Open circles are individuals fed on a high carbohydrate diet, and closed circles are individuals fed on a carbohydrate-free diet. Note that all the correlations shown are statistically significant ($P < 0.05$).

covariation due to nonspecific hydrolytic activity is accounted for. A multivariate ANOVA revealed a strong effect of diet on both axes for *Z. capensis* and *D. diuca* (Wilk's lambda = 0.48 and 0.40, respectively, $P < 0.01$). Thus, diet had a very strong effect on the nonspecific activity of intestinal enzymes in both *Z. capensis* and *D. diuca* (PCA axis 1, $F_{1, 13} = 9.1$ and $F_{1, 13} = 15.5$, respectively, $P < 0.01$). All of these enzyme levels were higher when birds were fed the carbohydrate-free diet. Diet also had a significant effect on the activity of aminopeptidase-N when the effect of diet on nonspecific modulation was removed (PCA axis 2, $F_{1, 13} = 7.2$ and $F_{1, 13} = 4.2$, respectively, $P < 0.05$).

Discussion

The levels of many digestive enzymes in vertebrates appear to be modulated reversibly with substrate levels (Stevens and Hume 1995). Recognizing that dietary modulation of the digestive enzymes and nutrient transporters of intestinal brush border membranes is widespread but not universal among vertebrates, Buddington et al. (1991) hypothesized a simple dichotomy. They proposed that digestive lability should be found in omnivorous species and absent in animals with little dietary variation, such as carnivores and dietary specialists. Karasov (1992) called this notion the "adaptive modulation hypothesis." In following paragraphs, we consider our results in the context of the adaptive modulation hypothesis. Specifically, we discuss whether the plasticity of digestive enzymes is correlated with dietary flexibility in birds, and we explore why disaccharidases appear not to be plastic in birds, whereas aminopeptidase-N seems to show significant specific modulation.

Is the Ability to Modulate Digestive Enzymes Correlated with Dietary Plasticity?

The adaptive modulation hypothesis has received little support from studies on bird digestive disaccharidases (Martínez del Rio et al. 1995). Birds show remarkably little variation in the expression of these enzymes when fed diets with contrasting carbohydrate content (reviewed by Karasov [1996]). Even species that are notoriously omnivorous, such as the European starling (*Sturnus vulgaris*) and the house sparrow (*Passer domesticus*), exhibit lack of plasticity in disaccharidase expression (Martínez del Rio et al. 1995; E. Caviedes-Vidal, D. Afik, C. Martínez del Rio, and W. H. Karasov, unpublished data). Surprisingly, all birds studied in the laboratory so far show significant increases in the membrane-bound intestinal oligopeptidase aminopeptidase-N when fed high-protein diets.

Our results differed from studies in other bird species. In the laboratory, the intestinal disaccharidases of *Zonotrichia capensis* and *Diuca diuca* did show a response to diets of contrasting composition. This response, however, was in the opposite direction from that expected from the adaptive modulation hypothesis. Both species showed increased maltase and sucrase activities when fed carbohydrate-free diets. A possible explanation for the increased disaccharidase levels found in birds fed a carbohydrate-free diet is that some of the variation in disaccharidase expression is the result of nonspecific induction (sensu Karasov and Diamond [1988]). Some diets may increase the activity of all enzymes. This hypothesis was supported by strong positive correlation among all enzymes in both *Z. capensis* and *D. diuca*. We found significant positive correlations among intestinal disaccharidases and aminopeptidase-N in *Z. capensis* and *D. diuca* in both the field and the laboratory.

The correlation between sucrase and maltase activity is not surprising. Maltase activity is the result of the action of sucrase-isomaltase and maltase-glucoamylase (Biviano et al. 1993). An increase in sucrase-isomaltase expression is necessarily translated also into increase in maltase activity. Furthermore, because sucrase-isomaltase and maltase-glucoamylase have overlapping specificities and presumably functions, it is conceivable that the same regulatory signals act on both of these digestive proteins (Afik et al. 1995). The correlation between the activity of aminopeptidase-N and the disaccharidases is perplexing, and we have no adequate explanation to account for it. Whatever mechanisms lead to this correlation, its existence casts doubt

on the hypothesis that intestinal digestive enzymes show tradeoffs (Diamond 1991).

Our multivariate analyses (PCA followed by multivariate ANOVA) provided additional support for the hypothesis that the carbohydrate-free diet elicited nonspecific modulation. These analyses also allowed discrimination between the nonspecific and the specific effects of diet on the expression of intestinal enzymes. When nonspecific levels of enzyme expression were accounted for, birds with higher intake of protein exhibited higher specific levels of aminopeptidase-N expression but no variation in disaccharidases.

Are Behavioral Flexibility and Physiological Plasticity Correlated?

Our field results suggested the hypothesis that in the controlled conditions of the laboratory, *D. diuca* would show little or no enzymatic plasticity, whereas *Z. capensis* would show significant plasticity. Contrary to our prediction, the responses of *Z. capensis* and *D. diuca* to our experimental diets were remarkably similar. Both species exhibited considerable plasticity in the expression of digestive enzymes. Although *D. diuca* showed exceedingly low variation in diet in the field, in the laboratory it was capable of responding to diets of contrasting composition. Because behavior is seemingly more plastic than physiology, conventional evolutionary wisdom asserts that an animal's first responses to a selective pressure are behavioral (Feder et al. 1987, p. 37). *D. diuca* provides a counterexample to this claim. In *D. diuca,* behavior appears to be less plastic than physiology.

Why Is Aminopeptidase-N Plastic Whereas Sucrase and Maltase Are Nonplastic?

Several studies have measured the effect of synthetic diets on the activity of the membrane-bound dipeptidase aminopetidase-N in birds (reviewed by Karasov [1996]). As predicted by the adaptive modulation hypothesis, all species studied show significant increases in the expression of aminopeptidase-N when fed diets with high protein content. Our field observations on *Z. capensis* and *D. diuca* support this generalization. Although these two species differed dramatically in dietary variation, their disaccharidase activities exhibited very similar variances. In contrast, the variance in aminopeptidase-N was twice as high in *Z. capensis* as in *D. diuca,* and thus it appeared to increase with increased dietary variation.

Why does the adaptive modulation hypothesis appear to apply to aminopeptidase-N, but not to disaccharidases? As do all conjectures, the adaptive modulation hypothesis relies on assumptions. Critical examination of these assumptions can help to understand why, in birds, disaccharidases appear to be relatively nonplastic, whereas their aminopeptidase-N responds readily to variation in diet. Some of the assumptions of the adaptive modulation hypothesis have been clearly recognized. Diamond (1991) used a two-tier, cost-benefit argument to explain the functional significance of substrate-induced modulation of intestinal enzymes. In brief, Diamond (1991) argued that natural selection maintains physiological plasticity in digestive proteins because (1) maintaining unused digestive enzymes and transporters can be costly in biosynthetic energy and limiting nutrients in the face of rapid turnover, and (2) unused proteins may take much-needed space in the presumably crowded apical membrane of intestinal cells.

The adaptive modulation hypothesis relies on a third assumption that to our knowledge has not been previously recognized. It assumes that intestinal enzymes and transporters can respond rapidly to changes in diet. If the time required for intestinal proteins to respond to a diet is long relative to the rate at which diet composition changes, the adaptive modulation hypothesis may not apply. The response of enzymes would lag behind a relatively rapidly changing diet composition. In the face of diets that change rapidly relative to the ability of enzymes and transporters to accommodate these changes, natural selection may favor constitutive levels that are constant, that may be not optimal for the composition of each individual dietary item, but that reflect the average intake of the nutrients hydrolyzed and transported by intestinal diet proteins. Using the parlance of evolutionary biology, if the response of an intestinal protein to changes in diet composition is relatively slow, then the dietary environment of animals is "fine grained" (sensu Levins [1968]), and natural selection should favor the expression of nonplasticity. In contrast, if the response of a digestive protein is rapid relative to changes in dietary composition, then the dietary environment is "coarse grained," and natural selection should favor plasticity. Padilla and Adolph (1996) describe a mathematical model that reaches conclusions very similar to those described in this paragraph. According to Padilla and Adolph (1996), inducible, reversible phenotypic plasticity need not be adaptive if the response of the organism to environmental changes shows a time delay.

Predicting whether digestive proteins such as enzymes and transporters are plastic or not may not only depend on the degree of omnivory of an animal, as hypothesized by Buddington et al. (1991), but also on the temporal scale at which these proteins respond to changes in the intake of their respective substrates. Information on the time responses of disaccharidases and aminopeptidase-N to changes in carbohydrate and protein intake is limited for vertebrates and almost nonexistent for birds (see Karasov and Hume [1997] for a review). In domestic chickens shifted from carbohydrate-containing to carbohydrate-free diets, it takes several days for disaccharidases to reach new equilibrium levels (Biviano et al. 1993). In contrast, rat intestinal cells induce aminopeptidase-N abruptly, within hours, when stimulated with a tetrapeptide substrate (Reisenauer and Gray 1985). We hypothesize that in birds, aminopeptidase-N shows rapid

induction, whereas the disaccharidases sucrase and maltase do not. Without data on the time course of induction of these enzymes, it is premature to implicate their variation in response time in the observed differences in lability between aminopeptidase-N and disaccharidases. However, we suspect that these differences may play an important role in explaining why some intestinal enzymes and transporters are substrate induced and others are not.

The response of birds to diets of contrasting composition in the laboratory and the field suggests that acceptance of the adaptive modulation hypothesis and of an association between digestive plasticity and omnivory is premature. Rejection, however, may also be inappropriate. The arguments outlined above suggest that the adaptive modulation hypothesis can be refined by including information on the time scale at which dietary shifts occur and on the time scale at which digestive proteins respond to these shifts. Knowledge about the physiological mechanisms that constrain the rates at which enzyme expression is modulated in response to environmental challenges can have evolutionary implications. It may hold the key to understanding why some enzymes and transporters show modulation, whereas others are constitutive.

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